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Suboptimal acclimation of photosynthesis to light in wheat canopies

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32 **One Sentence Summary:** High-resolution 3D reconstruction and ray tracing combined
33 with an empirical model of photosynthesis reveals sub-optimal photosynthetic
34 acclimation in wheat canopies.
35

Abstract

Photosynthetic acclimation (photoacclimation) is the process whereby leaves alter their morphology and/or biochemistry to optimise photosynthetic efficiency and productivity according to long-term changes in the light environment. Three-dimensional (3D) architecture of plant canopies impose complex light dynamics, but the drivers for photoacclimation in such fluctuating environments are poorly understood. A technique for high-resolution 3D reconstruction was combined with ray tracing to simulate a daily time course of radiation profiles for architecturally contrasting field-grown wheat canopies. An empirical model of photoacclimation was adapted to predict the optimal distribution of photosynthesis according to the fluctuating light patterns throughout the canopies. Whilst the photoacclimation model output showed good correlation with field-measured gas exchange data at the top of the canopy, it predicted a lower optimal light saturated rate of photosynthesis (P_{max}) at the base. Leaf Rubisco and protein content were consistent with the measured P_{max}. We conclude that although the photosynthetic capacity of leaves is high enough to exploit brief periods of high light within the canopy (particularly towards the base), the frequency and duration of such sunflecks are too small to make acclimation a viable strategy in terms of carbon gain. This suboptimal acclimation renders a large portion of residual photosynthetic capacity unused, and reduces photosynthetic nitrogen use efficiency (PNUE) at the canopy level with further implications for photosynthetic productivity. It is argued that (a) this represents an untapped source of photosynthetic potential and (b) canopy nitrogen could be lowered with no detriment to carbon gain or grain protein content.

Key Words

3D reconstruction, Canopy, Model, Nitrogen use efficiency, Photoacclimation, Photosynthesis, *Triticum aestivum* (Wheat)

Introduction

The arrangement of plant material in time and space can result in a heterogeneous and temporally unpredictable light environment. This is especially true within crop canopies, where leaf and stem architectural features can lead to complex patterns of light according to solar movement, weather and wind. This is likely to influence productivity because photosynthesis is highly responsive to changes in light intensity over short timescales (seconds to minutes). Leaf photosynthesis does not respond instantaneously to a sudden change in light level: the delay before steady state is reached is closely linked to the photosynthetic induction state, which is a physiological condition dependent on the leaf's recent 'light history' (Sassenrath-Cole and Pearcy 1994, Stegeman et al., 1999). Induction state is defined by factors including the activation state of photosynthetic enzymes (Yamori et al., 2012; Carmo-Silva and Salvucci, 2013), stomatal opening (Lawson and Blatt, 2014) and photoprotection (Hubbart et al., 2012). Together these determine the speed with which a leaf can respond to an increase, or decrease, in light intensity. It is thought that these processes are not always coordinated for optimal productivity in fluctuating light, as shown by the slow recovery of quantum efficiency for CO_2 assimilation (ϕCO_2) in low light (Zhu et al., 2004), high non-photochemical quenching (NPQ) during induction (Hubbart et al., 2012; Kromdijk et al., 2016) and slow stomatal opening and closure (Lawson and Blatt, 2014). It is predicted that such slow responses of photosynthesis to the environment can have a substantial impact on wheat yield (Taylor and Long, 2017).

The role of light-dependent changes in crop canopies has not had sufficient attention. Acclimation of photosynthesis to changes in light intensity and quality (here termed photoacclimation in order to distinguish it from acclimation to other environmental factors) is the process by which plants alter their structure and composition over long time periods (days and weeks), in response to the environment they experience. Photoacclimation can be broadly split into two types: photoacclimation that is determined during leaf development, including cell size and number plus leaf shape (Weston et al., 2000; Murchie et al., 2005) or photoacclimation that can occur within mature tissues (Anderson et al., 1995; Walters, 2005; Retkute et al., 2015). Whilst the former is largely irreversible, the latter, here termed dynamic photoacclimation, can be reversible. Differences include changes in light harvesting capacity (shown by chlorophyll a:b ratio), chlorophyll per unit nitrogen (N), electron transport capacity per

unit chlorophyll and rate of electron transport capacity relative to Rubisco activity (Björkman, 1981; Evans, 1989; Evans and Poorter, 2001). This involves change in relative amounts of a number of primary components and processes, including light harvesting pigment protein complexes (LHC), Calvin cycle enzymes and electron transport components such as the cytochrome b/f complex. It is normally considered that photoacclimation represents an economy of form and function, permitting higher capacity for carbon assimilation in high light whilst improving the quantum efficiency at low light (Björkman, 1981; Anderson and Osmund, 1987; Anderson et al., 1995; Murchie and Horton, 1997). This gives rise to the further concept that the plant must measure and predict changes in its environment to elicit the most efficient response. It is known that photoacclimation responses to fluctuating light can be complex (Vialet-Chabrand *et al.*, 2017) and that disruption of photoacclimation using mutants of *Arabidopsis thaliana* results in a loss of fitness (Athanasiou et al., 2010).

Is photoacclimation optimised for crop canopies? It is assumed to improve productivity because, following long-term shifts in light intensity, it permits a higher rate of photosynthesis at high light and a higher quantum efficiency at low light. Over time this will directly influence the ability of the canopy to 'convert' intercepted radiation to biomass and grain yield and reduce the amount of absorbed solar energy in potentially 'wasteful' processes such as non-photochemical quenching (Zhu et al 2010; Murchie and Reynolds, 2012; Kromdjik et al., 2016). However, this has never been empirically tested in crop canopies which often possess complex light dynamics that are dependent on architecture (Burgess et al., 2015). Hence, we do not know which features of photoacclimation would make appropriate traits for crop improvement.

To solve this problem, we need to first understand the features of natural light that trigger photoacclimation e.g. integrated light levels, duration of high - low light periods or the frequency of high - low light periods. Early work suggested that integrated PPFD could be an important driver (Chabot et al., 1979; Watling et al., 1997), however later work, using well characterised artificial fluctuations, highlighted the importance of the duration of high and low light periods (Yin and Johnson, 2000; Retkute et al., 2015). It therefore follows that the precise characteristics of the light environment are important when determining if photoacclimation is operating in a manner that maintains fitness and productivity. Past theoretical work has tended to focus upon canopies with randomly distributed leaves in space (Werner et al., 2001; Zhu et al., 2004) with few

recent models using more complex and realistic architectural features (Song et al., 2013; Burgess et al., 2015). This necessitates the study of photoacclimation in the context of light dynamics within accurately reconstructed 3-dimensional plant canopies because even moderate changes in architecture can have a large impact on light characteristics (Burgess et al., 2015). Photoacclimation to high light requires an energy source and resources (carbon (C), nitrogen (N) and others) in order to enhance, for example, Rubisco per unit leaf area. It can be argued that a high light saturated photosynthetic capacity (P_{max}) is advantageous under low light because it enables the exploitation of high light periods (sunflecks). However, maintenance of a thick high-light acclimated leaf with a high P_{max} (and high chlorophyll) may impose a respiratory burden and influence the efficiency of photosynthesis under low light. The advantage of maintaining a high P_{max} then becomes dependent on the frequency and duration of high sunflecks in the canopy and how fast photosynthetic induction can occur in response to each fleck. Although this question has been addressed to an extent in the ecological literature (e.g. Hikosaka, 2016) it is still not known whether there is an advantage to maintaining a higher P_{max} lower in the crop canopy in order to exploit sunflecks (Pearcy, 1990), or whether architecture influences the potential gain. Again, it depends on knowing the precise 3D pattern or light over time and predicting its likely effect on photoacclimation.

A last consideration concerns how photoacclimation is influenced by phenology and physiology within the canopy. In a cereal such as wheat, development occurs initially in high light, followed by progressive shading by younger leaves. Hence it might be expected that photoacclimation would track this change in light accurately. However, the photosynthetic system represents a significant sink for leaf N and other soil-derived mineral elements and this sink will increase in size as photosynthetic capacity of the leaf rises. It has been suggested that lower leaves in the canopy act as a functional reserve of minerals such as N. This may also lead to retention of a high P_{max} (Murchie et al., 2002; Sinclair and Sheehy, 1999). Lower leaves contribute relatively little to grain yield during grain filling (approximately 3% of light interception in leaf 4 at anthesis), thus optimising photoacclimation in flag leaf and second leaf will be the main targets for yield potential gains, whilst leaf 3 and 4 will be the main targets for gains in photosynthesis per unit N (PNUE). Although a decline in photosynthesis generally corresponds to the change in light during canopy development, there is variation in this relationship according to species (Hikosaka 2016). The extent of optimality of

photoacclimation (in isolation from other factors) depends on the exact sequence, frequency and duration of high light fluctuations of light within the canopy. The latter is unknown for realistic canopy light fluctuations. In other words, is it economically viable for a leaf to acclimate to high light in order to exploit brief periods of high light (Pearcy 1990)? We define optimality as that condition which results in the highest carbon gain for a given fluctuating light environment.

To address these questions, we have developed two novel techniques. First, a model of photoacclimation that provides a quantitative indicator of carbon gain, predicting optimal maximal photosynthetic capacity levels (P_{max}^{opt}) for a given variable environment (Retkute et al., 2015). Second, a method for the 3-dimensional (3D) high-resolution reconstruction of plant canopies without the need to parameterise structural models that, with available ray tracing techniques (Song et al., 2013), can characterise light in every point in the canopy over the course of a day (Pound et al., 2014; Burgess et al., 2015). This allows precise canopy architecture to be considered and a profile of light intensities for any part of the canopy throughout the day to be produced. Here we use these techniques in combination with manual measurements of photosynthesis to predict the optimal photoacclimation status (to light alone) throughout canopy depth according to the (variable) light environment determined by contrasting canopy architectures. We show that the P_{max} value optimised for light in all leaves in the bottom canopy layers is substantially lower than that measured, an observation that has implications for PNUE of the whole canopy and questions the common assumption that an accumulation of Rubisco at lower canopy positions allows exploitation of sunflecks.

Results

The Canopy Light Environment

Fig. 1 shows an example of the reconstruction process whilst Fig. 2 shows the final six canopies (three per growth stage) used within this study. The wheat lines selected were the same as those used for a previous study (Burgess et al., 2015) and chosen due to their contrasting architectural features. The Parent line (Ashby) contains more upright leaves, Line 2 (cv 23-74) more curled leaves and Line 1 (cv 32-129) with an intermediate phenotype (see materials and methods for more details on the wheat lines studied). Similar features were observed as in Burgess et al. (2015) except for a more curled leaf phenotype of Line 1 relative to the previous year, slightly increased plant height and altered Leaf Area Index (LAI; leaf area per unit ground area: see Table 1 and 2; measured physical plant measurements and reconstruction LAI values). Burgess et al. (2015) showed that manually measured leaf area corresponded well to reconstructed values. Here we find that LAI was slightly higher in all the reconstructions compared to the measured values, which was likely due to differences in the way in which stem and leaf area is accounted for in each method. In particular, the manual method did not account for all stem material (some was too large for the leaf area analyser) and the reconstruction method slightly over estimated stem area. This overestimation was consistent for all lines. Plant density, tillering and plant height were equivalent in Lines 1 and 2 but slightly higher in the Parent line (Table 1). Further architectural characteristics of the three contrasting lines are given in Supplementary Table S1.

Simulations of the light environment within each of the canopies indicate that the daily photosynthetic photon flux density (PPFD) decreases with depth in all three plots at both growth stages, however there is considerable heterogeneity at each depth that needs to be accounted for in the model application. Fig. 3 shows how PPFD varies with depth in three randomly selected triangles at each of the three depth positions where samples for rubisco measurements were taken and where gas exchange measurements were made. The progressive lowering in the canopy position also leads to more infrequent periods of high light intensity, or sunflecks, interspersed with periods of low light intensity, approaching the critical value for positive net photosynthesis (see below). Similar light signatures are seen for all canopies and both growth stages studied (data not shown). To validate the predicted light levels in each of the canopies using ray tracing, the modelled data were compared to manual measurements taken in the field

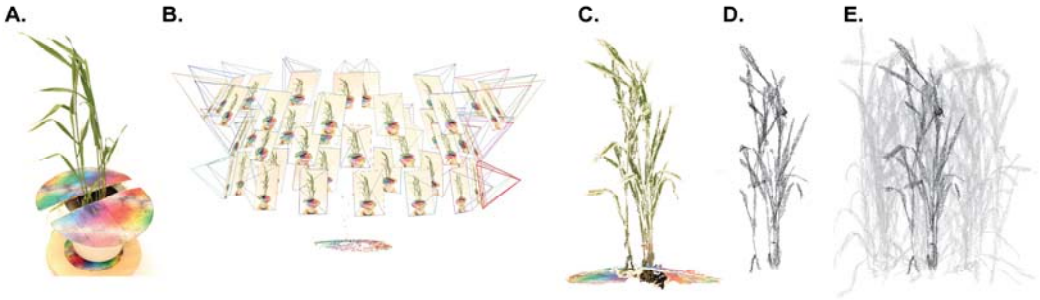

Figure 1. Overview of the reconstruction process **A.** original photograph, **B.** point cloud reconstruction using stereocameras (Wu, 2011), **C.** output point cloud, **D.** mesh following reconstruction method (Pound et al., 2014) and **E.** final canopy reconstruction. N.B. The multi-coloured disc in panels A-C is a calibration target, used to optimise the reconstruction process and scale the final reconstructions back to their original units.

with a ceptometer as the logarithm of the ratio of light received on a horizontal surface and light intercepted by a point on the leaf (Ln[L/Lo]; Supplementary Fig. S1).

Disparity between modelled and measured P_{max} at the bottom of the canopy

Fig. 4 shows light response curves of photosynthesis for each of the lines at three canopy levels. Typical responses are seen: a decline in both P_{max} and dark respiration rate with increasing canopy depth. A significant lowering of P_{max} was observed within the two lower layers at postanthesis. A comparison of photosynthesis rates with light levels (Fig. 3) shows that all leaves would remain above the light compensation point and positively contribute to carbon gain.

An empirical model of photoacclimation was applied (see Retkute et al*.,* 2015 and materials and methods) to predict the optimal P_{max} (P_{max}^{opt}) for 250 canopy positions. The model includes a time weighted average (τ); a calculation of the effect of a variable induction state which manifests as a gradually 'fading memory' of a high light event (see Materials and Methods: Modelling). The average is applied to the transition from low to high light (but not high to low) to effectively account for induction state which is very difficult to measure *in situ,* and not possible for all points in the canopy, as it reflects the past light history of the leaf. Within the main experiment of this study, τ was set at 0.2, which is equivalent to a maximum leaf memory of around 12 minutes, and is in line with previous studies and fit with past experimental data (Pearcy and Seemann, 1990; Retkute et al., 2015). The effect of this time weighted average is given in Supplementary Fig S2. Fig. 5 shows the result of the modelled P_{max}^{opt} against measured P_{max}. Strikingly, the measured P_{max} was substantially higher than predicted except in the upper parts of the canopy, which showed good correspondence. This was consistently

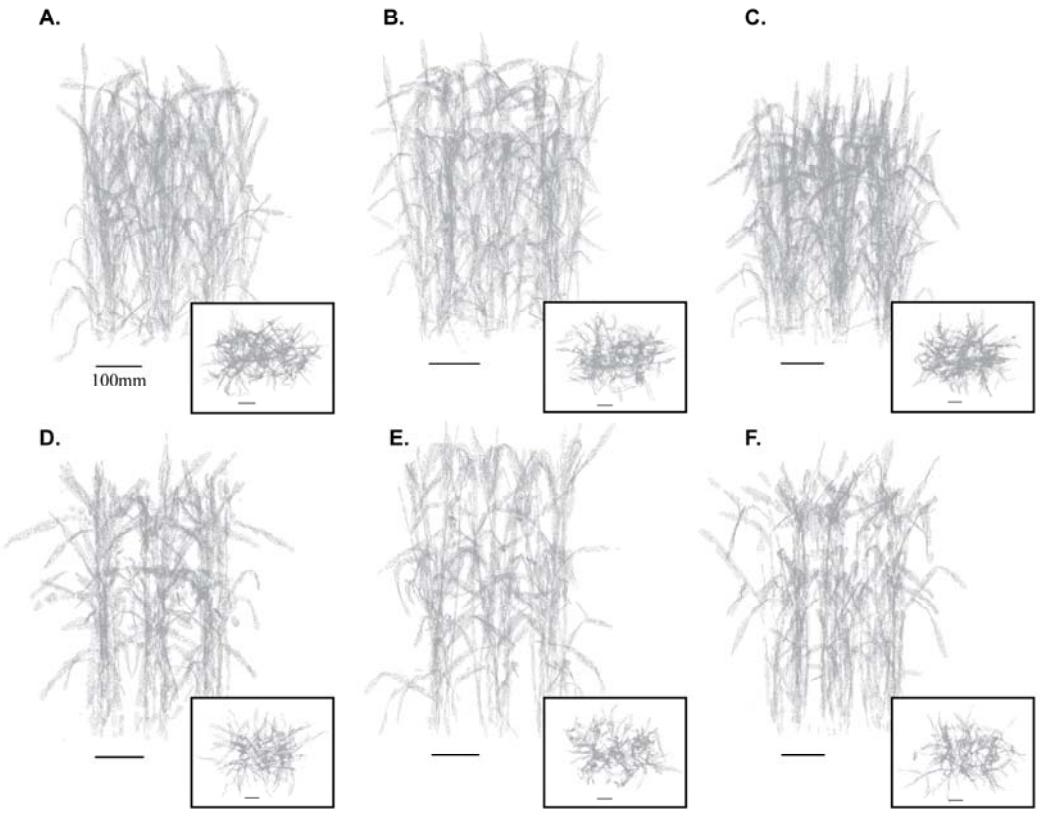

Figure 2. Example Canopy Reconstructions from front and top down views. **A-C.** Preanthesis and **D-F.** Postanthesis. **A, D.** Parent Line, **B, E.** Line 1 and **C, F.** Line 2

the case for all lines at both growth stages. In the lowest canopy positions (below 300 mm from the ground) the measured values of P_{max} were several times higher than the lowest predicted values: 1 – 2 μmol CO_2 m^{-2} s^{-1}. In these positions the important features were those that support a positive carbon gain in extremely low light environments notably a very low dark respiration level (measured at less than 0.5 μmol m^{-2} s^{-1}) and light compensation point. In other words, the measured P_{max} would rarely be achieved *in situ* largely due to the brevity of the high light periods and the slow induction of photosynthesis. A comparison with Fig. 3 shows that light levels in this part of the canopy were extremely low: 10 – 30 μmol m^{-2} s^{-1} punctuated by rare short lived high light events with a large variation in frequency and intensity. The decay of modelled P_{max}^{opt} was exponential (Fig. 5) consistent with that of light (Hirose, 2005) in contrast with the measured P_{max} which appeared linear. It was also notable that the different canopy architectures (analysed in Burgess et al., 2015 which used the same set of lines) were associated with similar disparity between measured and modelled levels of photosynthesis. However, this difference was greater in Line 2 (non-erect leaves) which had a higher rate of light extinction. A comparison of the modelled and measured P_{max} versus PPFD at 12:00 h, plus modelled P_{max}^{opt} versus daily PPFD is given in

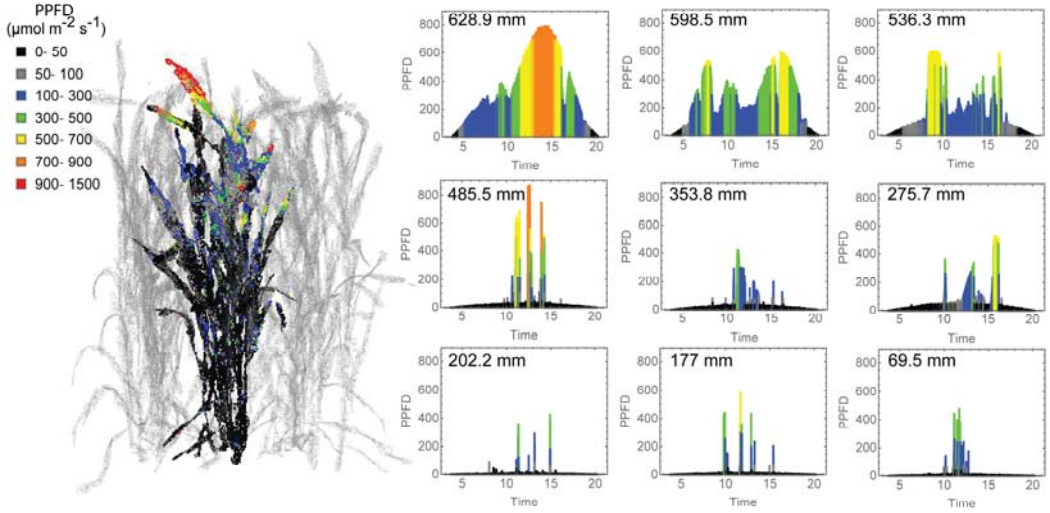

Figure 3. Progressive lowering of the canopy position in a canopy results in a reduction in daily integrated PPFD (μmol m^{-2} s^{-1}) but also the pattern and incidence of high light events within the canopy. The left hand panel shows a representative reconstructed preanthesis wheat canopy with a single plant in bold: Maximum PPFD ranges are colour coded. The right hand panels show PPFD during the course of a day at 9 representative and progressively lower canopy positions (the height of each canopy location from the ground given in the top left corner of each graph) calculated using ray tracing techniques.

Supplementary Fig. S3. This shows a similar spread of modelled versus measured P_{max} values and a linear relationship between modelled P_{max}^{opt} and daily PPFD. We also tested the model at a substantially lower value of τ (0.1, equivalent to a leaf memory of 6 minutes; Supplementary Fig. S4), which results in a more rapid response to light flecks. Even using this parameter, the P_{max} was substantially over estimated in the bottom layer of the canopy. A sensitivity analysis was performed based around the assumption of respiration being proportional to photosynthesis versus respiration having a linear relationship with respect to P_{max} (not allowing R_d vs P_{max} to pass through the origin). First, two lines were fitted to all measured data, and then we varied α by +/- 10%. In both cases changes in predicted P_{max} for light patterns at different layers in the canopy changed by less than 9% and could not account for the disparity between modelled and measured data.

Rubisco and protein content reflect measured, and not modelled, data

During canopy development wheat leaves will normally emerge into high light and then become progressively more shaded by production of subsequent leaves. The higher than expected measured P_{max} at the base of the canopy indicates retention of components of photosynthesis to a level that was excessive when compared to the prevailing light environment. The difference between measured and modelled P_{max} became progressively lower, moving from the bottom of the canopy to the top, until

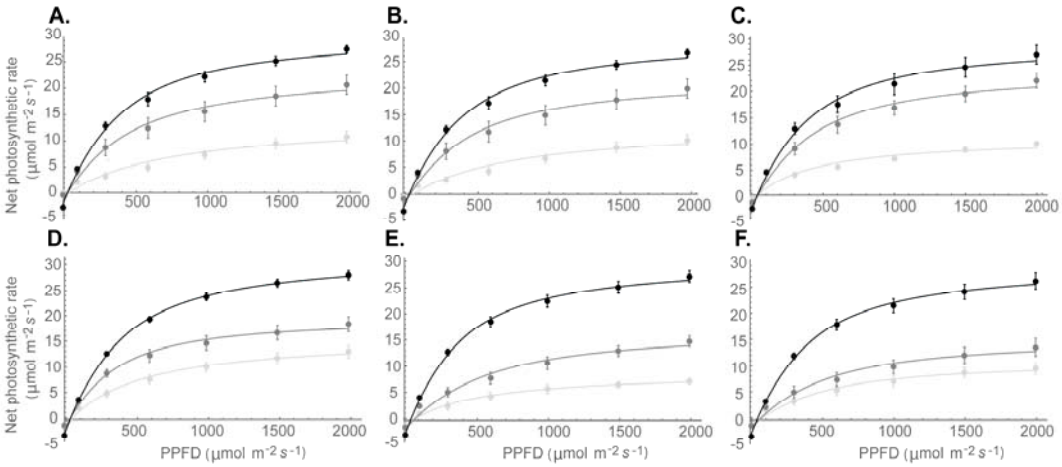

Figure 4. Fitted Light response curves for **A-C.** Preanthesis; Parent Line, Line 1 and Line 2, respectively. Layer top (black), middle (dark grey) and bottom (light grey). **D-F.** Postanthesis; Parent Line, Line 1 and Line 2, respectively. Layer top (black), middle (dark grey) and bottom (light grey).

there was complete correspondence at the top of the canopy. It is therefore important to confirm the activity of specific components of photosynthesis and compare them to both P_{max} and P_{max}^{opt} values. To understand how Rubisco activity might be changing we measured ACi responses and performed curve fitting to separate the maximum rate of carboxylation (V_{cmax}), electron transport (J) and end product limitation (TPU; see Table 3). V_{cmax} values at the top of the canopy are consistent with those observed in other studies (e.g. Theobald et al., 1998). As we descend the canopy V_{cmax} declines significantly (P<0.05) in a proportion that is consistent with measured, not modelled, P_{max}. Mesophyll conductance (G_m) was measured but showed no significant differences (P<0.05) between lines or layers.

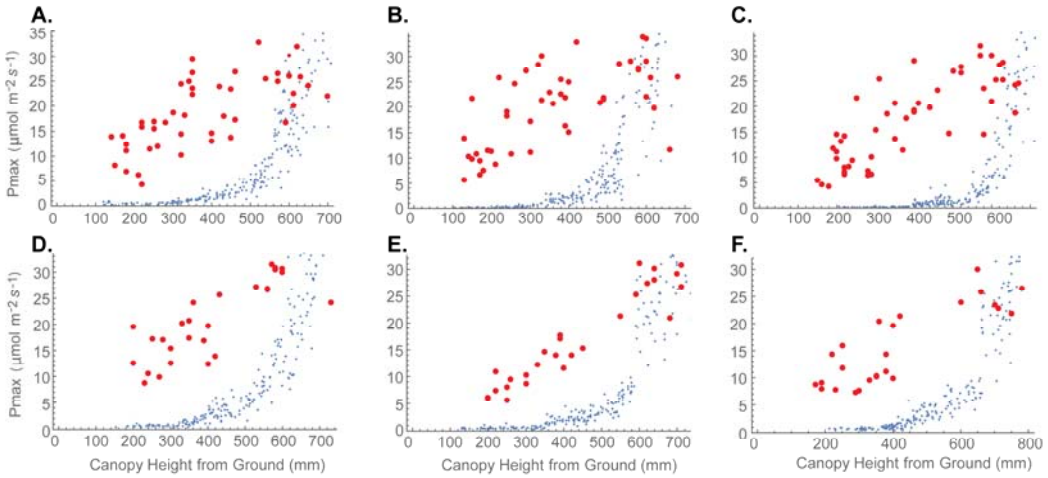

Figure 5. Whole canopy acclimation model output (blue) versus gas exchange measurement (red) graphs. The acclimation model was run at 250 locations throughout canopy depth to predict the optimal P_{max} at each location dependent upon the light environment that it experienced, calculated via ray tracing. The time weighted average (Eq. 4) was fixed at τ=0.2. This is an exponentially decaying weight used to represent the fact that photosynthesis is not able to respond instantaneously to a change in irradiance levels. If τ= 0 then a plant will able to instantaneously respond to a change in irradiance, whereas if τ>0 the time-weighted average light pattern will relax over the timescale τ. Model results are compared to field measured gas exchange. **A-C.** Preanthesis and **D-F.** Postanthesis. **A, D.** Parent Line, **B, E.** Line 1 and **C, F.** Line 2.

To analyse photoacclimation further, amounts of Rubisco, total soluble protein (TSP) and chlorophyll were quantified (Table 4). Rubisco amounts at the top of the canopy were consistent with those towards the upper end for wheat (e.g. Theobald et al., 1998) and are highly correlated with measured P_{max} and V_{cmax} within the canopy (Fig. 6). This indicates that Rubisco content accounts for all values of measured P_{max} and V_{cmax}, and not the modelled P_{max} values. Other work using similar techniques to characterise rice canopies came to a similar conclusion (Murchie et al., 2002). Chl a:b is a reliable indicator of dynamic photoacclimation i.e. fully reversible changes occurring at the biochemical level. The changes in Chl a:b are consistent with those expected for acclimation of light harvesting complexes (LHC) to a lower light intensity, with the lowered ratio indicating a greater investment into peripheral LHCII (Murchie and Horton, 1997). Interestingly the largest change in Chl a:b occurs in the upper half of the canopy where the greatest proportional change in light level occurs.

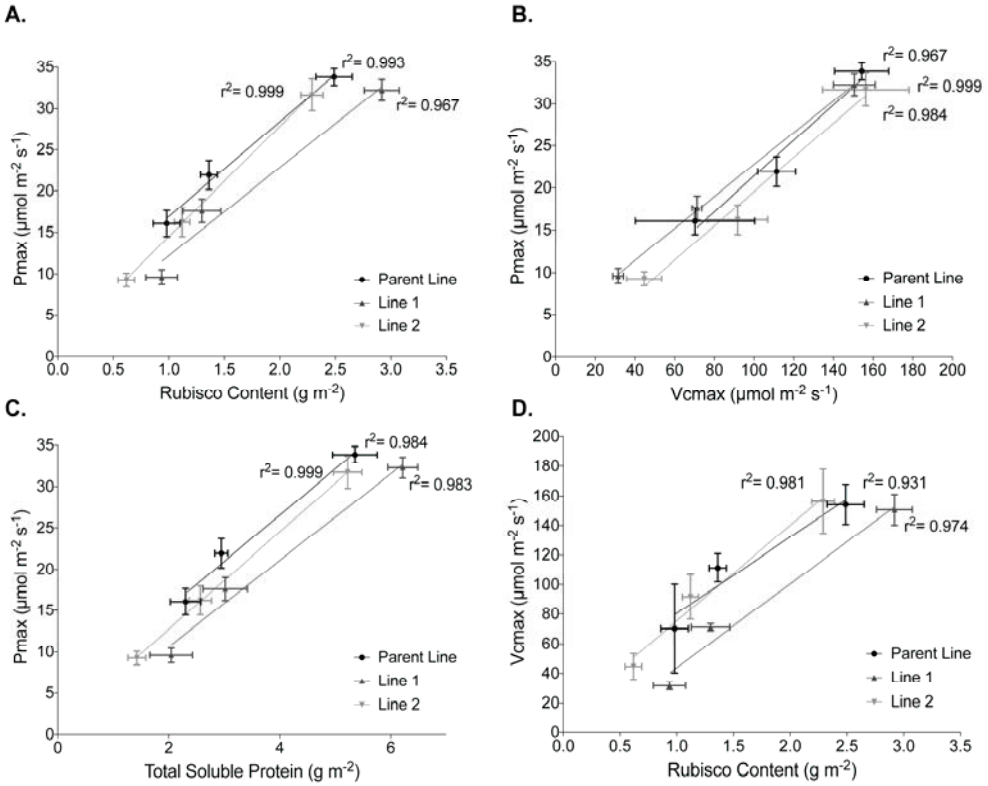

Figure 6. Relationships between photosynthesis (P_{max} taken from fitted light response curves) and Rubisco properties (V_{cmax} from fitted ACi curves and Rubisco/ total soluble protein (TSP) amount) throughout canopy depth, postanthesis; **A.** P_{max} and Rubisco content; **B.** P_{max} and V_{cmax}; **C.** P_{max} and Total Soluble Protein and; **D.** V_{cmax} and Rubisco content. Where black (round symbol) in the Parent Line, dark grey (triangle symbol) is Line 1 and light grey (upside down triangle symbol) is Line 2.

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Discussion

The regulatory aspects of photoacclimation and how it is triggered by changing light levels are little understood, but recent work has begun to address this and attempt to elucidate the link between variations in light and the resulting biomass and fitness (e.g. (Külheim *et al.*, 2002; Athanasiou *et al.*, 2010; Retkute *et al.*, 2015; Vialet-Chabrand *et al.*, 2017). In particular, the role of photoacclimation in determining productivity in crop canopies is not known. This paper takes a significant first step and reveals for the first time the relationship between highly realistic canopy architecture, the resulting dynamic light environment and its effect on photoacclimation. In addition to fundamental understanding of photoacclimation, this work has consequences in terms of nutrient usage within our agricultural systems, as discussed below.

Photosynthesis in nature responds largely to fluctuating light, not the unchanging or 'square waves' commonly used for studies in photoacclimation (Poorter *et al.*, 2016; Vialet-Chabrand *et al.*, 2017). The responses of leaves within a wheat canopy were analysed to predict the optimal state of photoacclimation using light history as a natural dynamic, rather than fixed or artificially fluctuating, parameter. To do this, a framework of image-based 3D canopy reconstruction and ray tracing combined with mathematical modelling was employed to predict the optimal distribution of photosynthetic acclimation states throughout a field grown wheat canopy based on the realistic dynamic light environment it experiences. The field measured and modelled data indicate two key features: (i) photosynthesis can vary greatly at the same canopy height according to both photoacclimation and instantaneous irradiance shifts and (ii) whilst the model indicates good correspondence to field data at the top of the canopy, the model consistently predicts lower optimal P_{max} values in the bottom canopy layers relative to measured data. These predictions are important because they consider the effects of fluctuating light in each layer. We conclude that the high light events at the base of the canopy are too short and infrequent to represent a substantial carbon resource for crop biomass. From this we conclude that plants are not optimising leaf composition in response to the long-term light levels they are experiencing, but rather are retaining excessive levels of photosynthetic enzymes at lower canopy levels. As discussed below the latter probably represents an intrinsic influence that could include developmental processes and nutrient remobilisation. Regardless of the cause it also signifies 'untapped' photosynthetic potential and opportunities to improve (photosynthetic) nutrient use efficiency.

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348 ***Influence of Canopy Light Dynamics on Acclimation***

349 Mono-species crop canopies have more consistent structural patterns in comparison
350 with natural systems, and are useful models for this type of work since data can be
351 classified according to stratification, but still include spatial complexity and an inherent
352 stochastic component. Photoacclimation according to canopy level is an expected
353 property (Supplementary Fig. S1). The dynamic nature of the in-canopy light
354 environment means that any leaf may be exposed to a range of conditions; from light-
355 saturation to light limitation, but with varying probability of either according to canopy
356 depth. Fig. 3 shows clearly how leaves at the top of the canopy experience high
357 likelihood of direct radiation with fluctuations ranging from two – three-fold depending
358 on leaf position. Lower in the canopy, occlusion results in an increasing dominance of
359 diffuse and low levels of radiation punctuated by brief and rare high light events
360 (sunflecks) that can be 10 – 50 times the mean level. Both the measured and modelled
361 canopy light levels indicate that the optimal photosynthesis should be low, based upon
362 the low, basal, levels of light the lower canopy layers receive. This is in agreement with
363 the modelled P_{max} values, however, the measured P_{max} values are much higher than this
364 (Fig. 5). The key question therefore is whether maintaining higher P_{max} is beneficial and
365 necessary to exploit sunflecks?
366
367 Much previous literature has discussed the importance of exploiting sunflecks as a
368 carbon resource in light-limited environments, such as forest understories (Pearcy,
369 1990) and the role of fluctuating light in determining photosynthesis – for which
370 nitrogen profiling in canopies has been discussed (Hikosaka, 2016). However, the
371 response seems to be variable, depending on physiological acclimation of each species
372 and stresses associated with increased temperatures and high light (Watling et al., 1997;
373 Leakey et al*.,* 2005). Here, the use of a novel acclimation model allows us to assess the
374 effectiveness of photoacclimation in terms of carbon gain at each position in realistic
375 canopy reconstructions. As sunflecks become rare in the lower portions of the canopy,
376 the model predicts that acclimation of P_{max} towards higher values becomes an
377 increasingly ineffective strategy in terms of exploiting them for carbon gain. To
378 efficiently exploit the sunflecks in the lower canopy positions, it is necessary to have a
379 high photosynthetic capacity (P_{max}), a rapid rate of photosynthetic induction and a
380 degree of photoprotective tolerance to avoid photoinhibition. The latter point is not

accounted for in this paper but has been noted in other species, especially where much higher leaf temperatures are involved (Leakey et al., 2005). Photoinhibition (Fv/Fm lower than 0.8) in lower parts of wheat canopies in the UK was not observed in this study (data not shown), or in a previous study (Burgess et al., 2015) and in our temperate system we do not expect excessive leaf temperatures. It is possible that high P_{max} observed in lower layers of the canopy help to prevent excessive photoinhibition. Photosynthetic induction state is determined by the previous light history of the leaf; by stomatal dynamics and the activation state of key enzymes such as Rubisco. Acclimation of P_{max} becomes more effective in terms of overall carbon gain where there is a lower frequency of light transitions but increasing duration of high light events (Retkute et al., 2015). This is consistent with the light data (Fig. 3), which shows rare, brief high light events lower in the wheat canopy.

Such very low levels of light within a crop canopy are comparable with forest floors where morphological and molecular adaptations are used to enhance light harvesting, carbon gain and avoid photoinhibition during high light periods (Powles and Bjorkman, 1981; Raven, 1994; Sheue et al., 2015). The interesting feature of cereal canopy development is the fact that leaves initially develop in high light and then are progressively shaded as the canopy matures. Since the morphology of the leaf is determined prior to emergence, all acclimation to low light, post emergence, must be at the biochemical level, as shown by the chlorophyll a:b ratio (Murchie et al., 2005). The low light levels within the wheat canopy also require effective acclimation of respiration rates to maintain positive carbon gain, and this was observed here (Fig. 4). Leaf respiration is a critical aspect of photoacclimation, permitting lowered light compensation points and positive carbon balance in low light. The relatively low rates of dark respiration in the lower layers and the very low measured light levels at the base of the canopy indicate that leaves maintain their (measured) high P_{max} alongside low respiration rates and light compensation points. Therefore, there must be some decoupling of P_{max} from these other photoacclimation processes at lower light levels. The importance of R_d should be stated here due to its importance in derivation of the term α and for confirmation that the same relationship between R_d and α holds regardless of the nature of the fluctuating light environment. However, first improvements must be made for the accurate measurement of R_d. This would also allow for detailed studies on the acclimation of R_d to a change in light levels.

We conclude, perhaps surprisingly, that the optimal strategy in lower parts of the wheat canopy where light is extremely low (<50 μmol m^{-2} s^{-1}) should not be geared towards exploiting sunflecks (previously seen as an important carbon resource) but towards light harvesting, maintenance of low leaf respiration and low light compensation point. Indeed, the photoacclimation of P_{max} to higher levels requires substantial investments of resources such as energy, N and C. It is still possible that the high measured P_{max} may allow a greater ability to exploit some sunflecks of increased duration where they do not lead to substantial photoinhibition (Raven, 2011). It is likely that the planting density has an effect: in this experiment, we have used standard sowing rates for the UK where the LAI is reasonably high leading to a dense canopy. The excessive accumulation of Rubisco in lower leaves may be more useful for exploiting light in planting systems where spacing is greater and light penetration is higher (Parry et al., 2010). There is little genetic variation for P_{max}, respiration rate and light compensation point in the three lines presented here (Fig. 4) although ongoing research is aimed at identifying further sources of genetic variation for improving these traits (Parry et al., 2010; Reynolds et al., 2012). Future studies will also need to focus on enhancing photoacclimation in flag leaf and L2.

Implications in terms of Nutrient Budgeting

The disparity between modelled data and manually measured data has consequences in terms of the canopy nutrient budget. Photosynthetic components are a significant sink for leaf N: chloroplasts account for up to 80 % of total leaf N, with Rubisco being the dominant enzyme (Makino and Osmond, 1991; Evans, 1989; Theobald et al., 1998). Higher photosynthetic capacity therefore requires a higher N (Evans and Terashima, 1987; Terashima and Evans, 1988; Verhoeven et al., 1997; Evans and Poorter, 2001; Terashima et al., 2005; Niinemets and Anten, 2009). Photoacclimation to high irradiance is often associated with an increase in the synthesis of Rubisco per unit leaf area (Evans and Terashima, 1987) and PNUE will therefore remain high only if the high irradiance is sustained. The decay of light within plant canopies commonly results in a correlation between distribution of photosynthetic capacity, light and specific leaf N (Anten et al., 1995; de Pury and Farquhar 1997; Hikosaka, 2016). However, in 'real' canopies the correlation is often not linear, leading to the conclusion that the relationship is suboptimal, either as an over-accumulation of N in lower regions of the canopy or an inability to photoacclimate to higher light (Buckley et al., 2013; Hikosaka,

2016). There appears to be species variation within these relationships: a recent meta-analysis showed that the N extinction coefficient for wheat was determined by LAI alone, whereas in other species it was co-determined by the light extinction coefficient (Moreau et al., 2012; Hikosaka, 2016). In the literature many other reasons have been given for this lack of correspondence including herbivory and stomatal and mesophyll limitation (Hikosaka, 2016). The novelty with the current work is the extent of disparity between predicted and optimal P_{max} *at most canopy levels*.

Wheat plants and other cereals exhibit a pattern of storage of N in leaves, leaf sheaths and stems prior to grain filling, whereby a substantial proportion of stored N is remobilised toward the grain where it contributes to protein synthesis (Foulkes and Murchie, 2011; Gaju et al., 2011; Moreau et al., 2012). For bread wheat, this is especially important for grain quality. Similar mechanisms occur in many plant species to conserve nutrients, therefore the retention of N in leaves represents a strategy for storage in the latter part of the plant life. Since wheat leaves develop in high light and become progressively shaded, their net lifetime contribution to canopy photosynthesis within the shaded environment will still be substantial. This secondary property of photosynthetic enzymes for N storage has been discussed previously e.g. Sinclair and Sheehy (1999). It is clear that this role is valid, but it is still not certain how it is effectively coordinated with photosynthetic productivity since remobilisation and subsequent senescence represent a compromise to canopy carbon gain in the latter grain filling periods. In this case, it is clear that the accumulation and retention of N in lower leaves of the wheat canopy is dominant over the regulation of key components of optimal photoacclimation, especially P_{max}, and it is doubtful whether the excess N is used to promote carbon gain at the canopy level. The mechanism for this partitioning 'strategy' is not known: it is still possible that the metabolic cost of removing the leaf N is simply greater than the cost of retaining it in the leaves. Were this to be the case then it implies a high degree of precision of the leaf photoacclimation process that is linked to whole plant metabolism. Therefore, questions must be raised as to the cost of this accumulation and whether all N is efficiently remobilised to improve grain quality. Recent data for UK wheat shows that only 76 % of leaf N is remobilised, indicating that a substantial improvement in NUE could be achieved with no penalty for photosynthesis or grain quality (Pask et al., 2012). However, this value is even lower for other plant components, with only 48% of N stored in the stem and 61% stored in the

leaf sheath remobilised to the grain (Pask et al., 2012). Altering the photoacclimation responses of the lower leaves to fluctuating light could bring about this improvement.

Cross-species correlations between leaf N content and dark respiration have been observed raising a further question over the respiratory cost of accumulating leaf N in such low light levels where the opportunities to exploit sunflecks are not high, nor are warranted in terms of photoacclimation of P_{max} (Reich et al.,1998). Sinclair and Sheehy (1999) pointed out that the erect nature of rice leaves had an important effect in terms of improving the capacity of the lower leaves to store N for remobilisation. Further, we suggest that even small changes in canopy architecture or physical properties (Burgess et al., 2015; 2016) would permit lower leaves to operate more efficiently as N storage organs in addition to their role as net carbon contributors.

Concluding remarks

Photosynthetic acclimation permits photosynthesis to optimise to the prevailing light conditions but its regulation in natural fluctuating light is poorly understood. Here we show that the accumulation of excessive photosynthetic capacity does not in fact allow exploitation of sunflecks for enhanced carbon gain, and is not optimal for exploiting the wheat canopy light environment as revealed by high resolution 3D reconstruction methods. This observation has some profound implications for the improvement of canopy photosynthesis and resource use efficiency in crops. First, the unused photosynthetic potential in lower parts of the canopy (which can be achieved without the addition of extra nutrients) could be used to enhance biomass and grain yield through increasing light penetration and reducing the inherent plant-plant competition. This can be achieved by previously published routes for example architecture (Burgess et al., 2015), by altering the distribution of chlorophyll content (Zhu et al., 2010; Ort & Melis, 2011) and/or by manipulating mechanical properties to optimise movement in response to low wind levels (Burgess et al., 2016). Second, there is an opportunity to improve photosynthetic nutrient use efficiency: we have shown that levels of canopy nutrients (especially N) could be reduced with no detrimental impact on either carbon gain or grain protein content.

Materials and Methods

Plant Material

Wheat lines with contrasting canopy architectures were selected from an ongoing field trial at the University of Nottingham farm (Sutton Bonington Campus), Leicestershire UK (52.834 N, 1.243 W) on a sandy loam soil type (Dunnington Heath Series) in 2015. 138 Double haploid (DH) lines were developed jointly by Nottingham and CIMMYT from a cross between the CIMMYT large-ear phenotype spring wheat advanced line LSP2 and UK winter wheat cultivar Rialto, as described in Burgess et al. (2015). This approach resulted in the formation of a large number of stable lines with contrasting canopy architecture but with values of light saturated photosynthesis consistent with previous published measurements for field grown wheat in the UK (Driever *et al.*, 2014; Gaju *et al.*, 2016). Two DH lines were then selected and each backcrossed three times with the UK spring wheat cultivar Ashby to produce BC_3 plants. The BC_3 lines were selected phenotypically to contrast for tillering and canopy architecture phenotypes. The BC_3 lines were then selfed for 5 generations before bulking seed of BC_3S_5 plants for the present trial. Three wheat lines were used for analysis: Ashby (the recurrent parent line), and two BC_3 lines, 32-129 (Line 1) and 23-74 (Line 2). This resulted in lines which were well adapted to the UK environment but which provided contrasts for canopy architecture.

The experiment used a completely randomised block design with three replicates. The plot size was 6.00 x 1.65 m and the sowing date was 20 October 2014. Previous cropping was winter oilseed rape. The field was ploughed and power harrowed and rolled after drilling. Seed rate was adjusted by genotype according to 1,000 grain weight to achieve a target seed rate of 300 seeds m^{-2}; rows were 0.13 m apart. 192 kg ha^{-1} nitrogen fertilizer as ammonium nitrate was applied in a three-split programme. P and K fertilizers were applied to ensure that these nutrients were not limiting. Plant growth regulator was applied at GS31 to reduce the risk of lodging. Herbicides, fungicides and pesticides were applied as required to minimise effects of weeds, diseases and pests. Two growth stages were analysed: preanthesis and postanthesis (equivalent to GS55-71; Zadoks et al*.,* 1974).

Plant Physical Measurements

Physical measurements were made on plants in the field (see Table 1 plus Supplementary Table S1). The number of plants and shoots within a 1 m section along the middle of each row were counted and averaged across the three replicate plots. This average value was used to calculate the planting density within the plots and thus used to ensure that the reconstructed canopies were representative of field conditions. Plant dry weight and area (excluding ears) was analysed by separating shoot material into stem and leaf sheath, flag leaf lamina and all other leaf lamina before passing them through a leaf area meter (LI3000C, Licor, Nebraska) for 6 replicate plants (2 per plot; those used for the reconstruction of canopies below). Each component was then dried individually in an oven at 80°C for 48 hours or until no more weight loss was noted. Plants were weighed immediately. Measured Leaf Area Index (leaf area per unit ground area: m^2; LAI) was calculated as the total area (leaf + stem) divided by the area of ground each plant covered (distance between rows x distance within rows) and averaged across the 6 replicate plants.

Imaging and Ray Tracing

3D analysis of plants was made according to the protocol of Pound et al. (2014) and further details are given in Burgess et al. (2015). An overview of this process is given in Fig. 1. From the sampled and reconstructed plants, canopies were made *in silico* according to Burgess et al. (2015). Two replicate plants representative of the morphology of each wheat line were taken per plot, giving 6 replicates per line, and reconstructed; at least 4 of these were used to form each the final canopies (Fig. 2). The wheat ears (present postanthesis) were manually removed from the resultant mesh as the reconstructing method is unable to accurately represent their form. Reconstructed canopies were formed by duplicating and randomly rotating the plants in a 3x4 grid, with 13 cm between rows and 5 cm within rows (calculated from field measurements). The LAI of each reconstructed canopy was calculated as the area of mesh inside the ray tracing boundaries divided by the ground area. The LAI of the plots were then compared to the LAI for each of the reconstruction plots; see Table 2. Total light per unit leaf area was predicted using a forward ray-tracing algorithm implemented in fastTracer (fastTracer version 3; PICB, Shanghai, China; Song et al., 2013). Latitude was set at 53 (for Sutton Bonington, UK), atmospheric transmittance 0.5, light reflectance 7.5%, light transmittance 7.5%, day 155 and 185 (4th June and 4th July: Preanthesis and Postanthesis respectively). FastTracer3 calculates light as direct, diffused and transmitted components

separately; these were combined to give a single irradiance levels for all canopy positions. The diurnal course of light intensities over a whole canopy was recorded in 1 minute intervals. The ray tracing boundaries were positioned within the outside plants to reduce boundary effects. To validate the light interception predicted by ray tracing, fractional interception was calculated at different depths throughout the field grown wheat canopies using a ceptometer (AccuPAR). Light levels at the top, three-quarters, half, quarter and bottom of the plant canopies were taken. Five replicates were taken per plot. This was compared with fractional interception calculated from ray tracing (Supplementary Fig. S1).

Gas Exchange and Fluorescence

Measurements were made on field grown wheat in plots in the same week in which the plants were imaged. For light response curves (LRC) and ACi response curves of photosynthesis, leaves were not dark-adapted. Leaf gas exchange measurements (LRC and ACi) were taken with a LI-COR 6400XT infra-red gas-exchange analyser (LI-COR, Nebraska). The block temperature was maintained at 20°C using a flow rate of 500 ml min^{-1}. Ambient field humidity was used. LRCs were measured over a series of 7 photosynthetically active radiation (PAR) values between 0 and 2000 μmol m^{-2} s^{-1}, with a minimum of 2 minutes and a maximum of 3 minutes at each light level moving from low to high. LRCs were measured at 3 different canopy heights; labelled top (flag leaf), middle and bottom, with height above ground being noted. Three replicates were taken per treatment plot per layer, thus leading to 9 replicates per line. Saturation of photosynthesis was verified for each light response step by conducting a separate set of light response curves where photosynthesis was logged every few seconds. It was verified that this protocol resulted in saturation at each light level. For the ACi curves, leaves were exposed to 1500 μmol m^{-2} sec^{-1}. They were placed in the chamber at 400 p.p.m. CO_2 for a maximum of 2 min and then CO_2 was reduced stepwise to 40 p.p.m. CO_2 was then increased to 1500 p.p.m., again in a stepwise manner. At least one replicate was taken per treatment plot per layer but with 5 replicates taken for each of the 3 lines. Individual ACi curves were fitted using the tool in Sharkey et al. (2007) with leaf temperature set at 20 °C, atmospheric pressure at 101 kPa, O_2 pressure at 21 kPa and limiting factors assigned as suggested in Sharkey et al. (2007). A Walz (Effeltrich, Germany) MiniPam fluorometer was used to measure dark-adapted values of Fv/Fm in the field wheat every hour between 09:00 and 17:00 h. 20 minutes dark adaptation was applied using the

610 method of Burgess et al. (2015). Four replicates were taken per plot per layer. Measurements
611 were not taken for the bottom layer.
612

613 **Rubisco quantification**

614 Leaf samples were taken from the same leaves and same region of the leaf as the gas
615 exchange measurements. One day was left between gas exchange and sampling. Leaf samples
616 (1.26 cm^2) were ground at 4°C in an ice-cold pestle and mortar containing 0.5 mL of 50 mM
617 Bicine-NaOH pH 8.2, 20 mM $MgCl_2$, 1 mM EDTA, 2 mM benzamidine, 5 mM ε-
618 aminocaproic acid, 50 mM 2-mercaptoethanol, 10 mM DTT, 1mM PMSF and 1% (v/v)
619 protease inhibitor cocktail (Sigma-Aldrich Co., St Louis, MO, USA). The homogenate was
620 clarified by centrifugation at 14700*g* and 4°C for 3 min. Rubisco in 150 µL of the supernatant
621 was quantified by the [^{14}C]-CABP binding assay (Parry et al., 1997), as described previously
622 (Carmo-Silva et al. 2010). The radioactivity due to [^{14}C]-CABP bound to Rubisco catalytic
623 sites was measured by liquid scintillation counting (PerkinElmer, Waltham, MA, USA). Total
624 soluble protein content in the supernatants was determined by the method of Bradford (1976)
625 using bovine serum albumin as a standard. Chlorophylls in 20 µL of the homogenate (prior to
626 centrifugation) were extracted in 95% ethanol for 4-8 hours in darkness (Lichtenthaler, 1987).
627 After clarifying the ethanol-extracted samples by centrifugation at 14000*g* for 3 min, the
628 absorbance of chlorophylls in ethanol was measured at 649 and 665 nm. Chlorophyll *a* and *b*
629 contents were estimated using the formulas $C_a = (13.36 \cdot A_{664}) - (5.19 \cdot A_{649})$ and $C_b = (27.43$
630 $\cdot A_{649}) - (8.12 \cdot A_{664})$.
631

632 **Modelling**

633 All modelling was carried out using Mathematica (Wolfram) using the techniques described
634 in more detail in Retkute et al., (2015) and Burgess et al., (2015). The acclimation model, here
635 adopted for use in the canopy setting, was originally developed based on the observation that
636 Arabidopsis thaliana plants subject to a fluctuating light pattern exhibit a higher P_{max} that
637 plants grown under a constant light pattern of the same average irradiance (Yin and Johnson,
638 2000; Athanasiou et al., 2010). The main model assumption is that plants will adjust P_{max}
639 from a range of possible values in such a way as to produce the largest amount of daily carbon
640 gain. The model predicts an optimal maximum photosynthetic capacity, P_{max}^{opt}, for a given
641 light pattern from light response curve parameters (ϕ, θ and α; explained below).
642

In this study, we sought to predict the maximum photosynthetic capacity, P_{max}^{opt}, as the P_{max} that represents maximal carbon gain at a single point within the canopy, based on the light pattern that point has experienced (i.e. using the light pattern output from ray tracing; as in right hand panel, Fig. 3). This was predicted across 250 canopy points, thus leading to distribution of P_{max}^{opt} values throughout each of the canopies. These 250 canopy positions (triangles) from each of the canopies were chosen as a subset of triangles that were of similar size (i.e. area) and constitute a representative sample distribution throughout canopy depth.

The net photosynthetic rate, P, as a function of PPFD, L, and maximum photosynthetic capacity, P_{max}, was calculated using the non-rectangular hyperbola (Eq. 1).

$$F_{NRH}(L, \phi, \theta, P_{max}, \alpha) = \frac{\phi L+(1+\alpha)P_{max}-\sqrt{(\phi L+(1+\alpha)P_{max})^2-4\theta\phi L(1+\alpha)P_{max}}}{2\theta} - \alpha P_{max} \quad (1)$$

Where L is the PPFD incident on a leaf (μmol m^{-2} s^{-1}), ϕ is the quantum use efficiency, θ is the convexity and α corresponds to the fraction of maximum photosynthetic capacity (P_{max}) used for dark respiration according to the relationship $Rd = \alpha P_{max}$ (Givnish, 1988; Niinemets and Tenhunen, 1997; Retkute et al., 2015). The value of α was obtained from the light response curves recorded in the field by fitting a line of best fit between measured P_{max} and R_d values for all individual plants (n > 20 plants for each wheat line and stage). Therefore, the relationship between P_{max} and Rd used in modelling is based on observation rather than assumption of linear fit. All other parameters (e.g. P_{max}, ϕ and θ) were estimated from the light response curves for three canopy layers using the Mathematica command **FindFit**.

As each canopy was divided into 3 layers, each triangle from the digital plant reconstruction was assigned to a particular layer, m, according to the triangle centre (i.e. with triangle centre between upper and lower limit of a layer depth). For each depth (d; distance from the highest point of the canopy), we found all triangles with centres lying above d (Eq. 2).

$$d_i = \max_{j=1,2,3;1\leq i\leq n} z_i^j - (z_i^1 + z_i^2 + z_i^3)/3 \quad (2)$$

675 Each triangle within a specific layer was assigned the light response curve parameters from
676 the corresponding measured data.
677
678 Carbon gain, C (mol m^{-2}) was calculated over the time period $t \in [0,T]$ (Eq. 3).
679
680 $$C(L(t), P_{max}) = \int_0^T P(L(t), P_{max})dt \quad (3)$$
681
682 Experimental data indicates that the response of photosynthesis to a change in irradiance is
683 not instantaneous and thus to incorporate this into the model Retkute et al. (2015) introduced
684 a time-weighted average for light (Eq. 4).
685
686 $$L_\tau(\mathrm{t}) = \frac{1}{\tau} \int_{-\infty}^{T} L(t')\, e^{-\frac{t-t'}{\tau}} dt' \quad (4)$$
687
688 This effectively accounts for photosynthetic induction state, which is very hard to quantify *in*
689 *situ* as it varies according to the light history of the leaf. The more time recently spent in high
690 light, the faster the induction response. The time-weighted average effectively acts as a
691 "fading memory" of the recent light pattern and uses an exponentially decaying weight. If $\tau =$
692 0 then a plant will able to instantaneously respond to a change in irradiance, whereas if $\tau > 0$
693 the time-weighted average light pattern will relax over the timescale τ. Within this study, τ
694 was fixed at 0.2 (unless otherwise stated) in agreement with previous studies and fit with past
695 experimental data (Pearcy and Seemann 1990, Retkute et al., 2015). The time-weighted
696 average only applies to the transition from low to high light. From the high to low, response
697 is here considered to be virtually instantaneous and the time-weighted average is not applied.
698 The effect of this decaying weight effectively acts as a "filter" for irradiance levels, with
699 photosynthesis as slow to respond from a transition from low to high light but quick to
700 respond following a drop in irradiance. This can be seen in Supplementary Fig. S3. The value
701 of τ (0.2) selected here represents a maximum leaf 'memory' of around 12 minutes that
702 exponentially declines according to time spent in the light. We verified this experimentally
703 using wheat leaves grown under irradiance levels that correspond to mid to upper canopy
704 level: induction from darkness to 1000 μmol m^{-2} s^{-1} typically took 10 – 20 minutes to reach
705 steady state rate. We also tested the model at a lower value of τ (0.1) to account for leaves
706 capable of faster induction or a longer 'memory' (Supplementary Fig. S4).

Tables

Table 1

Physical canopy measurements of each Genotype. The number of plants and tillers within a 1 m section along a row at the preanthesis stage were counted and averaged across 3 plots. The number of shoots for each of the plants used for reconstructions at preanthesis was counted. The resting plant height of 5 plants per plot was calculated. P value corresponds to ANOVA. Mean ± SEM, *n=3.*

Line	Average Number of Plants m^{-1}	Average Number of Shoots m^{-1}	Number of Shoots $plant^{-1}$	Average Resting Plant height (cm)	
				Preanthesis	Postanthesis
Parent	25.3±1.5	69.0±3.1	4.0±0.0	72.1±3.2	84.7±0.3
Line 1	21.3±3.2	61.0±2.3	3.5±0.3	68.3±2.0	90.7±1.6
Line 2	20.7±0.3	62.7±2.7	4.1±0.9	69.5±2.7	94.1±5.5
P value	0.287	0.170	0.675	0.579	0.063

Table 2

Plant and canopy area properties. Plants were separated into leaf and stem material and measured using a leaf area meter (LI3000C, Licor, Nebraska). Measured LAI was calculated as the total area (leaf + stem) divided by the area of ground each plant covered (distance between rows x distance within rows). The reconstructed LAI was calculated as mesh area inside the designated ray tracing boundaries (see Materials and Methods: Imaging and Ray Tracing). P value corresponds to ANOVA. Mean ± SEM, *n=3*

Line	**Measured (plant $^{-1}$)**				**Reconstruction**
	Leaf Area	Stem Area	Total Area	LAI	LAI
Parent	318±20	93±4	799±73	7.22±1.23	8.55
Line 1	312±27	66±10	807±42	6.71±1.30	8.39
Line 2	411±70	82±10	1118±113	8.78±1.90	9.75
P value	0.290	0.167	0.520	0.520	

718 **Table 3**

719 Parameters taken from curve fitting. P_{max} taken from light response curves and V_{cmax}, *J, TPU,* R_d *and* G_m taken from ACi curves (fitting at 25°C;

720 I= 3.74 using Sharkey et al*.,* 2007). Mean ± SEM, *n=9* for P_{max} and *n=5* for ACi parameters. P value corresponds to ANOVA.

	Line	Layer	P_{max} (µmol m^{-2} s^{-1})	V_{cmax} (µmol m^{-2} s^{-1})	J (µmol m^{-2} s^{-1})	TPU (µmol m^{-2} s^{-1})	R_d (µmol m^{-2} s^{-1})	G_m (µmol m^{-2} s^{-1} Pa^{-1})
Preanthesis	Parent	Top	30.1±2.2	225±14	305±5	24.0±0.4	5.1±0.5	12.3±7.5
		Middle	25.0±2.0	124±8	232±17	18.2±1.3	3.9±0.7	35.2±7.0
		Bottom	15.6±0.8	80±8	169±16	13.5±1.1	2.1±0.4	37.1±5.1
	Line 1	Top	32.3±0.7	185±19	313±24	24.2±1.9	5.4±1.1	28.1±8.2
		Middle	23.6±1.8	150±37	259±34	19.9±2.9	4.7±1.3	35.0±7.1
		Bottom	12.3±1.4	64±24	103±14	8.3±1.1	3.2±1.1	24.9±10.3
	Line 2	Top	30.3±2.5	200±46	290±24	23.1±2.5	4.2±2.2	37.3±4.9
		Middle	25.8±2.1	111±14	246±25	19.0±1.7	3.3±0.8	34.4±7.8
		Bottom	11.0±0.7	73±13	125±15	10.1±1.2	2.3±0.4	26.1±9.9
	P between Lines		0.638	0.733	0.718	0.691	0.380	0.772
	Mean	Top	30.9	203	303	23.7	4.90	25.9
		Middle	24.8	128	246	19.0	3.96	35.0
		Bottom	13.0	73	134	10.8	2.52	29.7
	P between layers		<0.001	<0.001	<0.001	<0.001	0.042	0.351
Postanthesis	Parent	Top	33.8±1.0	154±14	251±25	19.3±2.0	4.1±0.8	12.3±7.5
		Middle	21.9±1.8	111±10	207±20	16.1±1.6	2.7±0.3	26.9±8.7
		Bottom	16.1±1.6	70±30	106±19	8.6±1.4	1.8±0.5	26.5±9.6
	Line 1	Top	32.3±1.3	150±11	253±16	19.8±1.2	2.5±0.5	14.0±7.2
		Middle	17.6±1.4	71±2	132±6	10.3±0.5	1.2±0.2	36.0±6.2

	Bottom	9.6±0.9	31±3	65±7	5.4±0.4	1.3±0.2	28.0±8.6
Line 2	Top	31.7±1.9	156±22	262±15	20.7±0.9	4.1±0.7	17.8±7.3
	Middle	16.2±1.8	92±15	187±23	14.6±1.7	2.4±0.6	36.7±5.5
	Bottom	9.3±0.8	45±9	90±8	7.5±0.5	1.7±0.3	42.2±0.2
P between Lines		<0.001	0.106	0.027	0.024	0.012	0.009
Mean	Top	32.6	154	255	20.0	3.58	14.7
	Middle	18.5	92	175	13.7	2.08	33.2
	Bottom	11.7	50	87	7.1	1.60	30.7
P between Layers		<0.001	<0.001	<0.001	<0.001	<0.001	0.330

732 **Table 4**

733 Rubisco, total soluble protein and chlorophyll content plus chlorophyll a:b and Rubisco: chlorophyll ratios with each layer through the canopy at
734 the postanthesis stage. Means ± SEM, *n=6.* P value corresponds to ANOVA.

Line	Layer	Rubisco ($g\ m^{-2}$)	TSP ($g\ m^{-2}$)	Chlorophyll ($mg\ m^{-2}$)	Chlorophyll a:b	Rubisco : Chlorophyll
Parent	Top	2.49±0.16	5.35±0.40	844±49	1.93±0.04	2.95±0.11
	Middle	1.36±0.08	2.95±0.12	723±21	1.79±0.03	1.88±0.09
	Bottom	0.98±0.12	2.30±0.27	602±46	1.79±0.02	1.61±0.01
Line 1	Top	2.92±0.16	6.22±0.27	820±28	1.98±0.05	3.58±0.23
	Middle	1.30±0.17	3.02±0.40	667±39	1.79±0.02	1.92±0.15
	Bottom	0.94±0.14	2.04±0.38	532±55	1.68±0.03	1.74±0.16
Line 2	Top	2.29±0.10	5.22±0.26	734±36	1.99±0.04	3.13±0.10
	Middle	1.12±0.07	2.57±0.20	618±20	1.75±0.03	1.81±0.07
	Bottom	0.62±0.07	1.43±0.16	440±51	1.72±0.05	1.41±0.07
P between Lines		0.002	0.019	0.002	0.763	0.015
Mean	Top	2.57	5.60	799	1.96	3.22
	Middle	1.26	2.85	669	1.78	1.87
	Bottom	0.85	1.93	525	1.73	1.58
P between Layers		<0.001	<0.001	<0.001	<0.001	<0.001

Figure Legends

Figure 1: Overview of the reconstruction process A. original photograph, B. point cloud reconstruction using stereocameras (Wu, 2011), C. output point cloud, D. mesh following reconstruction method (Pound et al., 2014) and E. final canopy reconstruction. N.B. The multi-coloured disc in panels a-c is a calibration target, used to optimise the reconstruction process and scale the final reconstructions back to their original units.

Figure 2: Example Canopy Reconstructions from front and top down views. A-C. Preanthesis and D-F. Postanthesis. A, D. Parent Line, B, E. Line 1 and C, F. Line 2

Figure 3: Progressive lowering of the canopy position in a canopy results in a reduction in daily integrated PPFD (μmol m^{-2} s^{-1}) but also the pattern and incidence of high light events within the canopy. The left hand panel shows a representative reconstructed preanthesis wheat canopy with a single plant in bold: Maximum PPFD ranges are colour coded. The right hand panels show PPFD during the course of a day at 9 representative and progressively lower canopy positions (the height of each canopy location from the ground given in the top left corner of each graph) calculated using ray tracing techniques.

Figure 4: Fitted Light response curves for A-C. Preanthesis; Parent Line, Line 1 and Line 2, respectively. Layer top (black), middle (dark grey) and bottom (light grey). D-F. Postanthesis; Parent Line, Line 1 and Line 2, respectively. Layer top (black), middle (dark grey) and bottom (light grey).

760 **Figure 5:** Whole canopy acclimation model output (blue) versus gas exchange
761 measurement (red) graphs. The acclimation model was run at 250 locations throughout
762 canopy depth to predict the optimal P_{max} at each location dependent upon the light
763 environment that it experienced, calculated via ray tracing. The time weighted average
764 (Eq. 4) was fixed at τ=0.2. This is an exponentially decaying weight used to represent
765 the fact that photosynthesis is not able to respond instantaneously to a change in
766 irradiance levels. If τ= 0 then a plant will able to instantaneously respond to a change in
767 irradiance, whereas if τ>0 the time-weighted average light pattern will relax over the
768 timescale τ. Model results are compared to field measured gas exchange. A-C.
769 Preanthesis and D-F. Postanthesis. A, D. Parent Line, B, E. Line 1 and C, F. Line 2.
770

771 **Figure 6:** Relationships between photosynthesis (P_{max} taken from fitted light response
772 curves) and Rubisco properties (V_{cmax} from fitted ACi curves and Rubisco/ total soluble
773 protein (TSP) amount) throughout canopy depth; A. P_{max} and Rubisco content; B. P_{max}
774 and V_{cmax}; C. P_{max} and Total Soluble Protein and; D. V_{cmax} and Rubisco content. Where
775 black (round symbol) in the Parent Line, dark grey (triangle symbol) is Line 1 and light
776 grey (upside down triangle symbol) is Line 2.

Supplementary Data

Supplementary Table S1

Plant physiological measurements (plant height and leaf dimensions), preanthesis. Mean ± SEM, n=3.

Supplementary Figure S1: Experimental validation of the predicted light levels.
Supplementary Figure S2: Example of a time-weighted light pattern at τ=0.2 relative to a non-weighted line.
Supplementary Figure S3: Model output (blue) versus gas exchange measurement (red) graphs for the Parent Line, preanthesis.
Supplementary Figure S4: Whole canopy acclimation model output (blue) versus gas exchange measurement (red) graphs.

Supplementary Table S1

Plant physiological measurements (plant height and leaf dimensions), preanthesis. Mean ± SEM, n=3.

Supplementary Figure S1: Experimental validation of the predicted light levels. The logarithm of the ratio of the light received on a horizontal surface and light intercepted on a point on a leaf (Ln[L/Lo]) predicted by ray tracing (box and whisker) is compared to manual measurements made using a ceptometer (stars). Predicted and measured data for A. Parent Line, B. Line 1 and C. Line 2; top, middle and bottom layers of the canopy at 12:00 h.

Supplementary Figure S2: Example of a time-weighted light pattern at τ=0.2 (black line) relative to a non-weighted line (i.e. τ=0). Light patterns for A. top, B. middle and C. bottom canopy layers (as shown in Fig. 3). The time weighted average (Eq. 4) is an exponentially decaying weight used to represent the fact that photosynthesis is not able to respond instantaneously to a change in irradiance levels. If τ= 0 then a plant will able to instantaneously respond to a change in irradiance, whereas if τ>0 the time-weighted average light pattern will relax over the timescale τ. Within this study, τ was fixed at 0.2 unless otherwise stated.

Supplementary Figure S3: Model output (blue) versus gas exchange measurement (red) graphs for the Parent Line, preanthesis. A. P_{max} against the PPFD at 12:00 h.

Modelled PPFD is taken from the ray tracing output whereas measured PPFD is taken from ceptometer data in the field; N.B. ceptometer measurements were taken at a quarter, half and three quarters up the canopy, relating to bottom, middle and top layers, respectively, so the data was grouped accordingly. B. modelled daily integrated PPFD versus modelled P_{max}.

Supplementary Figure S4: Whole canopy acclimation model output (blue) versus gas exchange measurement (red) graphs. The acclimation model was run at 250 locations throughout canopy depth to predict the optimal P_{max} at each location dependent upon the light environment that it experienced, calculated via ray tracing. The time weighted average (Eq. 4) was fixed at τ=0.1. This is an exponentially decaying weight used to represent the fact that photosynthesis is not able to respond instantaneously to a change in irradiance levels. If τ= 0 then a plant will able to instantaneously respond to a change in irradiance, whereas if τ>0 the time-weighted average light pattern will relax over the timescale τ. Results shown for the Parent Line, Preanthesis.

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